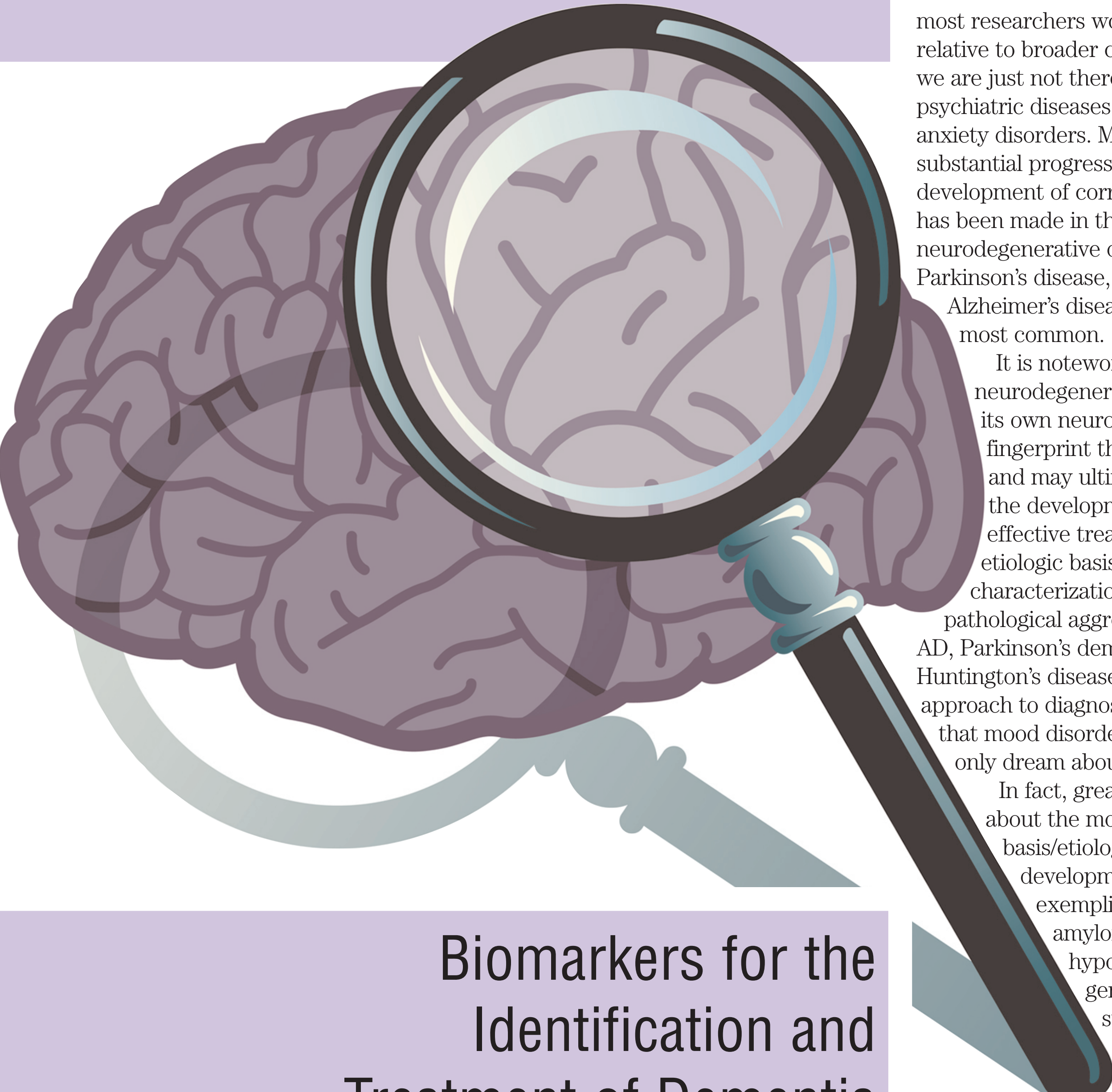

Biomarkers for the Identification and Treatment of Dementia

by Steven D. Targum, MD

INTRODUCTION

Biomarkers are objective measures that can potentially serve as indicators of neuroanatomical, physiological, or biological processes related to disease and/or direct indicators of pharmacological responses to therapeutic interventions.

Clinicians have long sought correlated biomarkers that would confirm diagnoses and facilitate treatment decisions. Evaluation of the utility of genetic, electrophysiologic, and/or biochemical markers for psychiatric disorders fills the pages of the most prestigious journals. But most researchers would agree that, relative to broader clinical application, we are just not there yet for psychiatric diseases like the mood and anxiety disorders. Meanwhile, substantial progress in the development of correlated biomarkers has been made in the area of neurodegenerative diseases (dementia, Parkinson's disease, etc), of which Alzheimer's disease (AD) is the most common.

It is noteworthy that each neurodegenerative disease has its own neuropathological fingerprint that distinguishes it and may ultimately facilitate the development of more effective treatments. Thus, the etiologic basis and biochemical characterization of the pathological aggregates found in AD, Parkinson's dementia, and Huntington's disease offer an objective approach to diagnostic differentiation that mood disorder specialists can only dream about.

In fact, greater understanding about the molecular basis/etiology for the development of AD, exemplified by the amyloid cascade hypothesis has generated novel drug strategies that may directly affect/disrupt the mechanism of disease progression. This article will briefly describe some of the current data suggesting that we are really on the verge of applying biomarkers for dementia in clinical practice.

WHAT ARE THE TELLTALE PATHOLOGICAL FINDINGS OF ALZHEIMER'S DISEASE?

In AD, there is a well documented extraneuronal accumulation/aggregation of β-amyloid fibrils into amyloid plaques and an intraneuronal accumulation of abnormal tau filaments as neurofibrillary tangles (NFT) and senile, neuritic plaques. In contrast, Parkinson's dementia is characterized by the accumulation of abnormal α-synuclein filaments (lewy bodies). There is some interconnection since more than 50 percent of AD patients reveal lewy bodies as well at autopsy.[1] Most researchers believe that amyloid plaques develop as a consequence of a progressive cascade of events resulting from abnormal breakdown of a larger protein, the amyloid precursor protein (APP).[1–4]

WHAT IS THE AMYLOID CASCADE HYPOTHESIS?

The accumulation of normally soluble β-amyloid, in particular the $A\beta_{42}$ peptide, in the brain initiates a cascade of events that promotes its conversion from soluble fibrils to insoluble oligomers that aggregate into fibrous Aβ masses that eventually become amyloid plaques. This amyloid cascade ultimately leads to neuronal dysfunction, neurodegeneration and cell death.[1]

As cognitive decline progresses in vulnerable individuals, the CSF levels of phospho-tau gradually rise and may be the most reliable biomarker of impending AD.[1,5–7]

The principal component of amyloid is the β-amyloid protein (Aβ), a 38–43 amino acid peptide formed from the extracellular domain of the amyloid precursor protein (APP). When APP is cleaved by an enzyme, called alpha-secretase, the resulting smaller protein does not develop amyloid plaques. However, γ- and β-secretase enzymes cleave longer forms of β-amyloid. Aβ40 is by far the most dominant form and is present in everyone as a single monomer. Several genetic mutations appear to influence APP processing and result in the production of higher levels of Aβ or longer Aβ-related peptides ($A\beta_{42}$ and $A\beta_{43}$). The longer forms of Aβ, particularly $A\beta_{42}$, are most susceptible to form amyloid fibrils that subsequently aggregate into oligomers (amyloid fibrillogenesis) and eventually become plaques.[1–3] Hence, the amyloid cascade hypothesis contends that abnormal metabolism of APP, influenced by genetic factors, leads to the development of toxic oligomers of Aβ that aggregate into extraneuronal plaques. As plaque develops and incorporate the $A\beta_{42}$ fibrils, CSF measurements actually reveal decreased $A\beta_{42}$ levels relative to normal, age-matched individuals. The resulting presence of amyloid deposits in extracellular areas of the amygdala, hippocampus, and neocortex represent a major feature of AD.

A strong support for the amyloid cascade hypothesis is that transgenic mice specifically bred to solely express a mutant human APP gene will develop amyloid plaques, and neuronal and microglial damage.[1] These mice are excellent animal models of AD and have become key players in the search for drugs that can interfere with the process. Novel drugs targeting β-amyloid can be tested in these transgenic mouse models prior to clinical trials.

WHAT IS TAU?

The tau protein is an intracelluar microtubule-associated protein that normally stabilizes microtubules in the cell cytoskeleton.[1–3] Under normal conditions, tau is regulated by phosphorylation. In AD, tau becomes hyperphosphorylated (phospho-tau) and accumulates as paired helical filaments that aggregate into masses inside the nerve cell bodies and dendrites as neurofibrillary tangles (NFT). NFT are the second major feature of AD. As cognitive decline progresses in vulnerable individuals, the CSF levels of phospho-tau gradually rise and may be the most reliable biomarker of impending AD.[1,5–7] In fact, many researchers also believe that the stability and reliability of phospho-tau measures make it the best biomarker candidate to discriminate between different forms of dementia.[5–8]

WHICH BIOMARKERS ARE BEING EVALUATED IN AD?

The range of biomarkers being studied in clinical trials is extensive. For instance, biomarkers in the cerebrospinal fluid (CSF) and plasma include β-amyloid 40 ($A\beta_{40}$), $A\beta_{42}$, total tau, and the three epitopes of phospho-tau (181,199, 231). Increased isoprostane and plasma homocysteine levels have been associated with increased risk for AD.[1] Isoprostanes are produced by oxidative stress and the formation of free radicals which have been implicated in the pathogenesis of AD. In addition, APOE genotyping is being tested in several clinical trials.

Recently, an international collaboration of researchers identified a panel of 18 signaling proteins that they believe can identify individuals at risk to develop AD.[9] Using archived plasma samples, they reported that the panel successfully classified plasma samples from AD patients 95 percent of the time and ruled out AD in 83 percent of plasma samples from control samples.[9]

There are also diverse approaches to brain imaging markers that can be anatomic (magnetic resonance imaging [MRI] measurement of regional brain atrophy), molecular (MRI protein aggregation imaging), metabolic (positron emission tomography [PET] 2-FDG and single-photon emission computed tomography [SPECT] or pathologic (PET visualization of Aβ aggregates using the Pittsburgh compound B.[3,8]

In 2005, a collaboration between NIH and private companies sponsored the Alzheimer's Disease Imaging Initiative (ADNI), which has consolidated the search for clinically useful biomarkers in an ambitious 800-subject, 50-site program being conducted in the United States and Canada.[1] ADNI will perform both MRI and PET imaging studies, obtain APOE genotyping, and collect CSF and plasma biomarkers including isoprostanes, tau, Aβ, sulfatides, and homocysteine.

Pathological CSF biomarkers are associated with progression from MCI to AD

Hansson et al (Lancet Neurol., 5: 228 -234, 2006)

Outcome 4 to 6 years	n	t-tau (ng/L)	p-tau $_{181}$ (ng/L)	Aβ42 (ng/L)	Aβ42/p-tau $_{181}$ ratio	APOE β4
Controls	39	326	61	700	12.5	10 (26%)
Stable -MCI	56	340	62	551	9.5	28 (50%)
MCI-AD	57	816	95	324	3.7	43 (75%)
MCI-other	21	480	60	579	10.7	6 (29%)

FIGURE 1.

IS THERE A GENETIC BASIS FOR AD?

Twin studies reveal that at least 80 percent of AD is inherited. However, less than 30 percent of AD genetics is solved. The known early-onset familial AD gene mutations in APP and the pre-senelins (PSEN1 and PSEN2) account for less than five percent of all AD.[1] These mutations contribute to an overproduction of β-amyloid. There are 10 new genetic association papers published each month, but most associations are never replicated. There is still only one established late-onset AD gene: APOE.

WHAT IS APOE? HOW DOES IT RELATE TO THE AMYLOID CASCADE HYPOTHESIS?

Apo-lipoprotein E is a gene on chromosome 19 that has been shown to bind to Aβ. There are three variants of APOE. Whereas the allele ε2 appears to be neuroprotective, the allele ε4 (APOE-ε4) has been shown to convey an inherited risk of approximately 50 percent for late-onset sporadic AD cases.[1,2,4] The rate of conversion from MCI to AD is significantly greater in ApoE-ε4 positive individuals.[1]

However, it is important to emphasize that not all cases of APOE-ε4 progress to AD. APOE-ε4 likely requires other inherited risk variants and environmental factors to trigger AD.

WHAT EVIDENCE EXISTS TO LINK β-AMYLOID AND TAU TO THE DEVELOPMENT OF AD?

Several studies have consistently reported an association between the β-amyloid and tau markers and progression of cognitive decline from mild cognitive impairment (MCI) to AD.[3,5–8] In one recent study, Hansson and colleagues showed that 42 percent of patients with MCI progressed to AD, 15 percent progressed to other forms of dementia, and 41 percent remained cognitively stable over a 4- to 7-year time interval.[5] Patients who developed AD had significantly increased total-tau and phospho-tau and decreased $A\beta_{42}$ relative to the controls and to MCI patients who remained stable or developed other forms of dementia (Figure 1).

Several independent studies have replicated these findings.[6–8] Fagan and colleagues followed 139 patients for 1 to 8 years and found that individuals with very mild or mild AD had reduced mean levels of CSF $A\beta_{42}$ and increased levels of CSF tau and phospho-tau (p-tau)181, consistent with other researchers.[6] Beyond that, the CSF $A\beta_{42}$ levels corresponded with the presence or absence of brain amyloid (imaged with Pittsburgh Compound B) in demented and nondemented individuals. They reported that the CSF tau/$A\beta_{42}$ ratio and p-tau (181)/$A\beta_{42}$ ratio predicted conversion from a Clinical Dementia Rating (CDR) of 0 (no cognitive impairment) to a CDR greater than 0 (MCI or greater).

Another study reported that a pre-defined cut-off value for one of the three epitopes of p-tau231 correctly classified conversion from MCI to AD in more than 80 percent of case across multiple sites.[7]

Thus, it appears that the very mildest symptomatic stage of AD exhibits the same CSF biomarker phenotype as more advanced AD, and that CSF phosphor-tau and tau/Aβ_{42} ratios may be useful preclinical biomarkers that predict future dementia in cognitively normal older adults.

These biomarkers could be useful in following treatment interventions as well as early identification of individuals at risk.

WHERE DO THE COMMERCIALLY AVAILABLE CHOLINESTERASE-INHIBITORS FIT IN WITH THESE BIOLOGICAL MARKERS?

The currently available cholinesterase-inhibitor drug therapies are based on the "cholinergic hypothesis" and suggest that AD is due to reduced biosynthesis of the neurotransmitter acetylcholine. In clinical practice, the acetylcholinsterase-inhibitors (AchE-I) only treat symptoms of the disease and have neither halted nor reversed it. Although these drugs provide some initial benefit, they do not affect the underlying molecular basis of AD and are therefore not neuroprotective. Instead, contemporary research has focused on whether or how to interfere with the toxic effects of the aggregated abnormal proteins, β-amyloid and tau. Essentially, there is no clinical value-added for measuring biomarkers during treatment with cholinesterase-inhibitors.

ARE CHOLINESTERASE INHIBITORS STILL USEFUL FOR THE TREATMENT OF ALZHEIMER'S DISEASE?

AChE-I can still offer short-term benefit for patients with AD and are often used in combination with the new drugs that are seeking disease modification. The objective of enhancing cholinergic transmission is still being pursued by some companies. For instance, Torrey Pines Therapeutics is developing novel muscarinic agonists while other companies explore the nicotinic acetylcholine receptor agonists. Nicotine enhances cognitive functions, such as learning, memory, and retention through activation of brain nicotinic acetylcholine receptors (nAChRs). Development of alpha[7] nicotinic agonists specifically may alleviate the cholinergic deficit present in AD without the undesirable side effects caused by over activation of other addictive nicotinic receptors or muscarinic receptors involved in cardiovascular or gastrointestinal functions (e.g., nausea). These side effects limit the doses of AChE-I tolerated and thus the level of cholinergic activation mediated by these compounds.

Memory Pharmaceuticals, Targacept, and EnVivo are developing programs of selective, orally active alpha-7 nicotinic receptor agonists for the treatment of AD. Memory Pharmaceuticals recently reported success with MEM3454, their lead nicotinic alpha-7 receptor partial agonist in an eight-week, 80 patient study. Subjects receiving either 5mg or 15mg of MEM3454 achieved a statistically significant effect on the Quality of Episodic Secondary Memory (QESM) test compared to placebo (p=0.023 and p=0.050, respectively). Targacept has reported that their lead alpha7 nicotinic compound, isopronicline (TC5619), may also be neuroprotective as demonstrated by the prevention of apoptosis (cell death) in animal models. A second Targacept compound, TC-1734 (AZD3480), a highly selective alpha4 beta2 nicotinic receptor agonist recently showed positive results in a Phase II-clinical trial of age associated memory impairment (AAMI).

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WHAT ABOUT MEMANTINE, AN NMDA INHIBITOR?

Research suggesting the involvement of glutamatergic neuronal excitotoxicity in AD led to the development and introduction of memantine, a novel NMDA receptor antagonist that has been shown to be moderately effective in AD. Memantine apparently inhibits the prolonged influx of Ca2+ ions which forms the basis of neuronal excitotoxicity and preserves the physiological function of the receptor.[10]

Some researchers believe memantine may have neuroprotective properties that could be disease-modifying, but there is still no clinical evidence that memantine can protect against NMDA receptor-mediated excitotoxicity

However, some recent animal studies have reported that NMDA receptor antagonists can block the uptake and internalization of β-amyloid peptide into cultured hippocampal cells and can protect against neurotoxicity associated with intracerebroventricular administration of ß-amyloid *in vivo*.[10]

Additional studies exploring the potential neuroprotective, disease-modifying features of NMDA receptor antagonists are underway. Meanwhile, memantine is often used in combination with other drugs to slow the progression of AD.

WHAT NEW DRUGS ARE BEING DEVELOPED TO INTERFERE WITH THE AMYLOID CASCADE?

The current hypothesis regarding the development of AD suggests that the toxic element in the etiology of AD is actually not the plaque, but an earlier form of Aβ, which is neither the soluble Aβ monomer nor the final, aggregated polymer, but an intermediate oligomeric species.[1,3] Consequently, much drug development efforts have focused on compounds that would inhibit fibrillization or prevent the formation of oligomeric species.

There are several potential drug treatments for AD currently undergoing clinical trials. Perhaps, the most interesting trials are examining strategies that directly challenge the progression of the amyloid cascade. Hence, drugs that impede the cleavage of β-amyloid into the more toxic longer forms or inhibit aggregation of Aβ fibrils may alter disease progression. I'll give a few examples of drugs already in well publicized clinical trials.

Tarenflurbil (MPC-7869, formerly R-flubiprofen) is a gamma-secretase modulator sometimes called a selective $A\beta_{42}$ lowering agent. Tarenflurbil is believed to reduce the production of the toxic $A\beta_{42}$ in favor of shorter forms of the peptide.

Another drug, tramiprosate (3APS or Alzhemed), is a GAG-mimetic molecule that is believed to work by binding soluble Aβ and thereby prevent the accumulation of the toxic Aβ oligomers. The initial Phase 3 trial for Alzhemed was not successful for cognitive measures, but a second trial is underway in Europe. The initial study did measure CSF biomarkers. A regression analysis performed on Aβ CSF concentration changes as a function of Alzhemed dose revealed a linear relation (p=0.041) between the decreases in Aβ 42 concentrations and dose. Subjects who received 3APS 150mg BID (the highest of three tested doses) had a decrease in mean CSF $A\beta_{42}$ concentration versus baseline (p=0.017). However, there were no significant differences in mean CSF T-tau concentrations between the baseline and three-month follow-up assessments for any of the treatment groups.

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A recent, small, double-blind, placebo-controlled study of a gamma-secretase inhibitor (Eli Lilly) treated 51 patients with mild to moderate AD for 14 weeks. Although no treatment-related changes were seen in group cognitive or functional measures, plasma Aβ 40 levels decreased by 58.2 percent in the 100mg group and by 64.6 percent in the 140mg group, and plasma Aβ 42 decreased to below assay sensitivity after the last treatment.

Epix recently announced positive findings from a two-week exploratory study with PRX-01340, a 5-HT-4 receptor agonist in which 150mg per day yielded a significant improvement in the cognition measure (ADAS-cog) versus the placebo group (p<0.005). Significant improvement in ADAS-cog measures are not typically observed in less than 12 weeks of therapy. Of note, the combination arm (PRX-01340 with donepezil) was not statistically significant. This drug is purported to work by increasing acetylcholine release, secreting soluble APP, and possibly inhibiting secretion of Aβ.

Another compound, PBT2 (Prana Biotechnology) has just completed a small clinical trial in AD. PBT2 works by interfering with the aggregation of Aβ fibrils by disrupting the requisite metal binding by copper and zinc. In transgenic mouse studies, PBT2 significantly reduced amyloid plaque as well as soluble and insoluble Aβ. In this study, plasma and CSF measures of Aβ40 and 42, tau, and phospho-tau were obtained at baseline and endpoint in all of the patients. The results are due in early 2008.

Vaccines or immunotherapy for AD are also being developed. By training the immune system to recognize and attack Aβ, the immune system might reverse deposition of amyloid and thus stop the disease. The trials of Elan's AN-1792 were abruptly stopped in 2002 when it was reported that six percent of multidosed participants (18 of 300) developed symptoms resembling meningoencephalitis.[11] In follow-up of the patients who had participated, approximately 20 percent developed high levels of antibodies to

Aβ and some showed slower progression of the disease, maintaining memory-test levels while placebo patients worsened. Less toxic forms of the vaccine are now being tested. However, safety is still a concern since microcerebral hemorrhages with passive immunization and meningoencephalitis with active immunization are still potential risks.

WHAT IS ON THE HORIZON?

Actually, we are on the horizon. Unlike current efforts for mood and anxiety disorders, contemporary clinical trials for dementia invariably include biomarker measures in their research.

The ADNI program mentioned earlier will provide biomarker and imaging (MRI and PET) data as they become available. ADNI is currently enrolling 200 normal subjects, 400 patients with mild cognitive impairment (MCI), and 200 subjects with mild AD. The ADNI protocol will test several specific hypotheses based on the clinical and biomarker data. A few examples include the following: The rate of conversion from MCI to AD will average 10 to 15 percent per year; baseline scores on logical memory APOE-ε4 status will predict conversion from MCI to AD; plasma isoprostanes will be related to disease severity and higher levels will predict a faster rate of decline; and hippocampal volume and posterior cingulate glucose metabolic rate will predict rate of decline and conversion from MCI to AD.[1]

Although invasive, the application of CSF biomarkers (particularly phospho-tau and $A\beta_{42}$) has already become part of drug development protocols. The recent report of 18 signaling proteins that can differentiate AD patients from controls and possibly predict individuals at risk offers even broader clinical application.[9] Given these definitive examples, it is clear that the incorporation of specific biomarker measurements for assessing dementia will become a standard of care within the next few years.

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